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**APPENDIX A**

**Clean Copy of Pending Claims**

1. A method of predicting the *in vivo* cytotoxicity of a chemical compound comprising:
  - a) culturing cells in culture medium that comprises a plurality of concentrations of said chemical compound;
  - b) measuring a first indicator of cell health at four or more concentrations of said chemical compound;
  - c) measuring a second indicator of cell health at four or more concentrations of said chemical compound;
  - d) measuring a third indicator of cell health at four or more concentrations of said chemical compound;
  - e) predicting a toxic concentration ( $C_{tox}$ ) of said chemical compound from the measurements of steps (b), (c) and (d).
2. The method of claim 1, wherein each said first, second and third indicators is independently selected from the group consisting of indicators of cellular replication, indicators of mitochondrial function, indicators of intracellular energy balance, indicators of cell membrane integrity and indicators of cell mortality.
3. The method of claim 1, wherein said predicting comprises:
  - i) performing dose-response analyses of measurements from steps (b), (c) and (d);
  - ii) identifying from the dose response analyses the highest concentration of the chemical compound at which a measurable toxic effect of the chemical compound is not observable (NOEL) in any dose response analyses of step (i);
  - iii) selecting as  $C_{tox}$  a concentration less than or equal to the concentration identified in step (ii).
4. The method of claim 1, further comprising determining a concentration of said compound that produces a half maximal toxic effect ( $TC_{50}$ ) for each of said indicators of cell health.
5. The method of claim 1, wherein said plurality of concentrations are selected from a concentration range from 0 micromolar and to about 300 micromolar.
6. The method of claim 3, wherein said plurality of concentrations includes at least two concentration values above the  $C_{tox}$  concentration.
7. The method of claim 3, wherein step (i) comprises plotting the measurements for each said cell health indicators on a graph as a function of concentration for each said cell health indicators of the chemical compound.
8. The method of claim 7, wherein the measurements of each of said cell health indicators are expressed relative to a control measurement as a function of concentration of the chemical compound.

9. The method of claim 8, wherein the measurements of all of said cell health indicators are plotted on a single graph.
10. The method of claim 1, wherein at least one of said cell health indicators is measured from the supernatant of said cell culture.
11. The method of claim 10, wherein at least one of said cell health indicators is measured from cellular components of said cell culture.
12. The method of claim 1, wherein said first health indicator monitors cellular replication, said second cell health indicator monitors mitochondrial function, and said third cell health indicator monitors membrane integrity.
13. The method of claim 12, wherein cellular replication is monitored with an assay selected from the group consisting of an assay that measures  $^3\text{H}$ -thymidine incorporation; a BrdU incorporation assay, or a CYQUANT® assay.
14. The method claim 13, wherein said mitochondrial function is monitored with an assay selected from the group consisting of an ATP assay, an MTT assay, an Alamar Blue assay, and a Rhodamine 123 assay.
15. The method of claim 14, wherein said membrane integrity is monitored with an assay selected from the group consisting of a glutathione S-transferase assay, lactate dehydrogenase assay, aspartyl aminotransferase assay, alanine aminotransferase assay, isocitrate dehydrogenase assay, sorbitol dehydrogenase assay, glutamate dehydrogenase assay, ornithine carbamyl transferase assay,  $\gamma$ -glutamyl transferase assay, and alkaline phosphatase assay.
16. The method of claim 15, further comprising measuring a fourth cell health indicator selected from the group consisting of indicators of cellular replication, indicators of mitochondrial function, indicators of intracellular energy balance, indicators of cell membrane integrity and indicators of cell mortality.
17. The method of claim 16, wherein said fourth indicator of cell health is an indicator of energy balance that is measured with an assay selected from the group consisting of an ATP/ADP balance assay and oxygen consumption assay.
18. The method of claim 16, wherein the fourth indicator of cell health is a cell mortality assay selected from the group consisting of cell number assay and an apoptosis assay.
19. The method of claim 16, further comprising measuring a fifth cell health indicator selected from the group consisting of indicators of cellular replication, indicators of mitochondrial function, indicators of intracellular energy balance, indicators of cell membrane integrity and indicators of cell mortality.

20. The method of claim 1, wherein the culturing is for a period of at least 72 hours, and wherein the predicting comprises:
  - (i) performing dose-response analyses of measurements from steps (b), (c), and (d);
  - (ii) determining a concentration of said compound that produces half-maximal toxic effect (TC<sub>50</sub>) for each of said indicators of cell health; and
  - (iii) selecting as C<sub>tox</sub> a concentration less than or equal to the lowest TC<sub>50</sub> concentration determined in step (ii).
21. The method of claim 1, wherein said cells are primary cells.
22. The method of claim 1, wherein said cells are of mammalian origin and are selected from the group consisting of liver cells, kidney cells, brain cells, fibroblast cells, nerve cells, skin cells, lung cells, spleen cells, endometrial cells, cardiac cells, stomach cells, breast cells, stem cells and a hematopoietic cell; and cell lines derived from any of these cells.
23. The method of claim 1, wherein said cells are from a mammalian cell line.
24. The method of claim 23, wherein said cells are liver cell line cells.
25. The method of claim 24, wherein said liver cells are human liver cell line cells.
26. The method of claim 24, wherein said liver cells are rodent liver cell line cells.
27. The method of claim 1, wherein said chemical compound is selected from the group consisting of an antimicrobial agent, an antitumor agent, an immunomodulator, a neurotransmitter, an agent for treatment or prevention of a central nervous system disease or disorder or cardiovascular disease or disorder; and an anti-inflammatory agent.
28. A method of developing an agent for treating a disease or disorder comprising:
  - a) assaying a plurality of compounds for a biological activity correlating with a desired therapeutic effect;
  - b) selecting one or more compounds with the desired biological activity;
  - c) predicting *in vivo* cytotoxicity of the compounds from step (b) according to claim 1;
  - d) selecting a compound with acceptably low levels of predicted cytotoxicity; and
  - e) testing said compound for efficacy against said disease or disorder.
29. The method of claim 28, further comprising a step, after said selecting step (d), of manufacturing a composition comprising the selected compound in a pharmaceutically acceptable diluent or carrier.
30. The method of claim 28, wherein said disease or disorder is selected from the group consisting of pain; diseases and disorders of the central nervous system, cancer, diabetes, depression, immunodeficiency diseases, autoimmune diseases and disorders,

gastrointestinal diseases and disorders, cardiovascular diseases and disorders, inflammatory diseases and disorders; and infections.

31. A method of identifying a lead compound for drug development comprising
  - a) obtaining a library of compounds having a potential therapeutic activity;
  - b) analyzing said library to identify compounds that are predicted to be cytotoxic, said analysis comprising predicting the cytotoxicity of the compounds according to claim 1; and
  - c) selecting a compound from said library that has an acceptably low level of predicted cytotoxicity.
32. The method of claim 31, further comprising a step, after said selecting step (c), of manufacturing a composition comprising the selected compound in a pharmaceutically acceptable diluent or carrier.
33. The method of claim 31, wherein the compounds in said library share common structural features.
34. A method of screening chemical compounds to select candidate therapeutic agents, comprising:
  - a) performing an *in vitro* activity assay to determine concentrations of chemical compounds required to achieve an activity ( $C_{ther}$ ), wherein the activity correlates with a desired therapeutic effect *in vivo*;
  - b) predicting cytotoxicity of the compounds according to claim 1; and
  - c) selecting as candidate therapeutic agents compounds having a  $C_{ther}$  less than  $C_{tox}$ .
35. The method of claim 34, further comprising a step, after said selecting step (c), of manufacturing a composition comprising the selected compound in a pharmaceutically acceptable diluent or carrier.
36. A method of prioritizing candidate therapeutic agents for pharmaceutical research and development comprising:
  - a) performing an *in vitro* activity assay to determine concentrations of chemical compounds required to achieve an activity ( $C_{ther}$ ), wherein the activity correlates with a desired therapeutic effect *in vivo*;
  - b) predicting cytotoxicity of the compounds according to claim 1;
  - c) determining the ratio of  $C_{tox}:C_{ther}$  for each compound to provide an Estimated Therapeutic Index (ETI) for each compound; and
  - d) prioritizing the compounds as candidate therapeutic agents from the ETIs wherein a higher ETI correlates with a higher priority for further development.
37. A method of predicting the *in vivo* cytotoxicity of a chemical compound comprising:
  - a) culturing cells in culture medium that comprises a plurality of concentrations of a chemical compound;

- b) measuring a first indicator of cell health at four or more concentrations of said chemical compound;
  - c) measuring a second indicator of cell health at four or more concentrations of said chemical compound;
  - d) measuring a third indicator of cell health at four or more concentrations of said chemical compound;
  - e) predicting a cytotoxic mechanism by which the chemical compound exerts cytotoxic effects from the measurements of steps (b), (c) and (d).
38. A method according to claim 37, wherein step (e) further comprises predicting a toxic concentration ( $C_{tox}$ ) of said chemical compound from the measurements of steps (b), (c) and (d).